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PROVISIONAL SPECIFICATION

Invention Title: TERMITE ATTRACTANT AND/OR FEEDING STIMULANT COMPOUNDS

The invention is described in the following statement:

GH REF: P50322AD/BJN

TERMITE ATTRACTANT COMPOUNDS

The present invention is concerned with termite attractant and/or feeding stimulant compounds and, more particularly, with termite attractant and/or feeding stimulant compounds for use in a termite bait.

Organochlorines have underpinned termite control around the world including in Australia, for many decades. With the ban on the use of organochlorines for termite control in Australia since 1995 and earlier or at similar times in other countries, increasing efforts are being mounted to develop alternative termite management systems. Bait systems for the control of active termite infestations are considered increasingly the key management option for such situations.

In bait systems termites are offered a matrix on which the insects ought to feed in preference to other food sources available to a termite colony. Termites either take up a slow-acting, non-repellent lethal product which is incorporated into the food (matrix) or the termites which aggregate in the matrix are directly treated with such a product. In both scenarios the agent is transported into the nest by the foragers and there distributed throughout the colony either via food exchange or mutual grooming between nest mates.

Following considerable research around the world there is now a growing awareness that just finding an effective bait toxin, initially thought to be the main impediment to the application of baits, is no guarantee at all that a bait system will work effectively in practice. Control strategies relying on baits have to cope with the fact that termites have a choice and that the insects cannot be forced to make contact with the baits. Termites have to be able to locate a bait station in the first place, and once it is found, be attracted to it in significant numbers so that adequate transfer of the toxin from the bait site to the colony can occur. Differences in behaviour between species of termite, between colonies within a species and between conditions

at various sites potentially restrict the effectiveness of this control strategy. Currently used bait matrices, in most cases just straight cellulose products (timber, cardboard, paper), do not ensure contact and build up of termite numbers in bait stations in a reliable, predictable fashion.

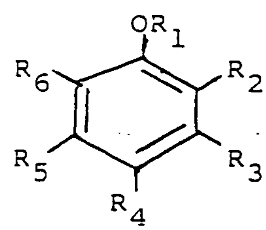
Termites are social insects and the social organisation of termite colonies largely depends on chemical signals present in the environment or produced by members of the colony. These signals modulate a variety of behaviours including foraging for food or communal exploitation of a food source. For example, during feeding, termites release a chemical signal from an exocrine gland that stimulates nest mates to feed at the same site, thereby ensuring a rapid and efficient exploitation of the food source.

All species of termite have paired labial glands located in the thorax. The glandular ducts join in the head with those of the water sacs and the contents are secreted from the mouth as saliva. This secretion has been reported to have various functions depending on the species, and has variously been identified as a defensive substance in soldier termites, a regulator of nest microclimate, a supporter of fungal cultivation in the nest or as a social nutrient. In addition, the labial glands have been said to secrete a cementing substance for nest construction or gallery building and have been identified as a source of digestive enzymes.

More recently, Reinhard et al., *Journal of Chemical Ecology, Vol. 23 No. 10, 1997* concluded that the labial gland secretion may play a pheromonal role during food exploitation, and that this might be a general phenomenon in termites. Reinhard et al. took labial gland extracts and used these in feeding choice tests. They observed that the labial gland secretion carries a signal that stimulates gnawing and feeding by termite workers during food exploitation. The extract of the labial gland even elicited feeding behaviour when applied without food onto

glass plates. These extracts were tested with both *Reticulitermes santonensis* and *Schedorhinotermes lamanianus* and proved to elicit a significant feeding preference in the two species. In view of this, Reinhard et al. suggested that the signal function of the labial gland secretion for food exploitation is phylogenetically old and non-species specific. The chemical signal has now been identified for the first time and has proved to work as a powerful feeding stimulant at natural low concentrations on a wide range of termite species. In view of this a class of compounds which stimulate termite feeding has been identified.

According to a first aspect of the present invention there is provided a termite attractant and/or feeding stimulant compound for stimulating termite feeding having the following general formula I:



(I)

wherein R_1 is selected from the group consisting of hydrogen, alkyl and substituted alkyl;

R_2, R_3, R_4, R_5 and R_6 are independently selected from the group consisting of hydrogen, hydroxyl, alkoxy or substituted alkoxy, or R_2 and R_3 together, R_3 and R_4 together, R_4 and R_5 together and/or R_5 and R_6 together form an aryl group;

provided only that at least one of R_2, R_3, R_4, R_5 or R_6 is hydroxyl, alkoxy or substituted alkoxy.

Preferably, R_1 is hydrogen or alkyl and R_2, R_3, R_4, R_5 and R_6 are independently selected from hydrogen, hydroxyl or alkoxy. Typically at least three of R_2, R_3, R_4, R_5 and R_6 are hydrogen.

In a particularly preferred embodiment of the present invention the compounds of formula I are selected

from para-hydroquinone (1,4-dihydroxybenzene), catechol (1,2-dihydroxybenzene), resorcinol (1,3-dihydroxybenezene), phloroglucinol (1,3,5-trihydroxybenzene), 4-methoxyphenol, methoxyhydroquinone (2,5-dihydroxyanisole) and 1,4-dimethoxybenzene. Moreover, addition compounds such as quinhydrone (an addition compound of 1 mol hydroquinone and 1 mol quinone) may be used, as well as other quinhydrones.

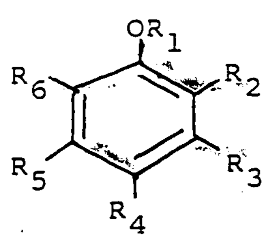
As used throughout the specification the term "alkyl" refers to straight or branched chain alkyl radicals, preferably C_1-C_{10} alkyl radicals and, more preferably, C_{1-4} alkyl radicals.

As used throughout the specification the term "substituted alkyl" refers to an alkyl radical substituted by any substituent, conveniently, by hydroxyl, alkoxy, carboxy, carboxyalkyl, carbamoyl, carbamido, amino, mono- or di- alkyl substituted amino, halogen, alkylcarbonyloxy or alkylcarbonylamino.

As used throughout the specification the term "aryl" refers to a six-membered carbocyclic aromatic ring or a five- or six-membered heterocyclic aromatic ring containing 1, 2 or 3 oxygen, nitrogen or sulphur atoms as the heteroatom, and includes fused ring systems containing a plurality of such rings.

According to a second aspect of the present invention there is provided a method of attracting and/or stimulating feeding activity in termites, comprising the steps of:

(1) providing a compound of general formula I



(I)

wherein R_1 is selected from the group consisting of

hydrogen, alkyl and substituted alkyl;

R_2, R_3, R_4, R_5 and R_6 are independently selected from the group consisting of hydrogen, hydroxyl, alkoxy or substituted alkoxy, or R_2 and R_3 together, R_3 and R_4 together, R_4 and R_5 together and/or R_5 and R_6 together form an aryl group;

provided only that at least one of R_2, R_3, R_4, R_5 or R_6 is hydroxyl, alkoxy or substituted alkoxy;

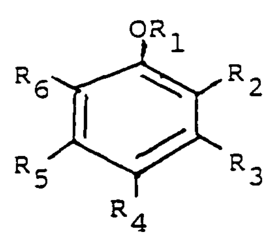
(2) applying said compound of general formula I to a locus.

The compounds of general formula I act as an attractant to termite species, in particular, to *Mastotermes darwiniensis, Coptotermes acinaciformis, Kalotermes flavicollis, Cryptotermes brevis, Hodotermes mossambicus, Zootermopsis angusticollis, Reticulitermes flavipes, Reticulitermes santonensis, Heterotermes indicola, Schedorhinotermes lamanianus, Coptotermes formosanus, Nasutitermes nigriceps, Nasutitermes exitiosus, Trinervitermes trinervoides* and *Macrotermes subhyalinus.*

According to a third aspect of the present invention there is provided a bait for attracting and/or stimulating feeding activity in termites, comprising:

(1) a food source; and

(2) a compound of general formula I:



(I)

wherein R_1 is selected from the group consisting of hydrogen, alkyl or substituted alkyl;

R_2, R_3, R_4, R_5 and R_6 are independently selected from the group consisting of hydrogen, hydroxyl, alkoxy or substituted alkoxy, or R_2 and R_3 together, R_3 and R_4

together, R_4 and R_5 together and/or R_5 and R_6 together form an aryl group;

provided only that at least one of R_2, R_3, R_4, R_5 or R_6 is hydroxyl, alkoxy or substituted alkoxy.

Typically the food source is a source of cellulose such as paper, cardboard, canite, chipboard, and sound or fungally decayed wood. The compound of general formula I is applied to the bait matrix in any convenient manner, such as by spraying a solution of the compound on the bait matrix, soaking the bait matrix in such a solution or by admixture with a solid compound of general formula I.

The bait matrix may also contain synergists and other attractants, as well as beneficial components such as nitrogen-containing compounds, carbohydrates and the like as nutrients.

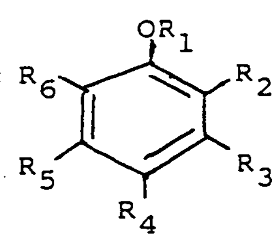
Where necessary, antioxidants such as BHT, BHA or tocopherols may be added to stabilise the active compound within the bait. A controlled release system for the compound of general formula I may be employed where desirable.

Preferably, the bait matrix includes added toxins such as chitin synthesis inhibitors, insect growth regulators and other termiticides. Alternatively, termiticidal substances can be applied to the bait matrix once it has been deployed in the field and has attracted a significant number of termites. In either case, it is preferred that the toxin be slow-acting and non-repellant so as to be transported into the nest by foragers and there distributed throughout the colony either via food exchange or mutual grooming between the nest mates.

According to a fourth aspect of the present invention there is provided a termiticidal composition comprising:

(1) a termiticidal substance; and

(2) a compound of general formula I:



(I)

wherein R_1 is selected from the group consisting of hydrogen, alkyl or substituted alkyl;

R_2, R_3, R_4, R_5 and R_6 are independently selected from the group consisting of hydrogen, hydroxyl, alkoxy or substituted alkoxy, or R_2 and R_3 together, R_3 and R_4 together, R_4 and R_5 together and/or R_5 and R_6 together form an aryl group;

provided only that at least one of R_2, R_3, R_4, R_5 or R_6 is hydroxyl, alkoxy or substituted alkoxy.

It has been found that para-hydroxyquinone is the natural feeding stimulant, but exists in the labial glands of termites almost entirely as its glucose conjugate, 4-hydroxyphenyl-ß-D-glycokyranoside, which is commonly called β-arbutin. β-arbutin and glucose conjugates of the other compounds of general formula I may also be used in the invention described above. In particular, β-arbutin or glucose conjugates of the other compounds of formula I can be incorporated into a bait matrix and, through slow decay generating an active compound of general formula I, could act as a slow-release system.

Preferred embodiments of the invention will now be described, by way of example only, with reference to the following examples.

Example 1 - Use of Labial Glands Extracts as Termite Attractants

In order to prepare labial gland extracts, termites were killed and the paired labial glands were removed. The labial glands were disrupted by freezing them for 15 minutes at -20°C and extracted with 0.6 ml of water for 12 hours at room temperature. Then the extract was frozen at -20°C until used. The labial gland extracts prepared and tested are listed in Table I. Each extract was chemically analysed for the pressence of para-hydroquinone, and it was found to be present in all. Selected extracts were used in a bioassay to establish feeding choice, as indicated in Table 1, below.

Table 1: Labial gland extracts prepared and tested

termite species	no. of glands extracted	chemically analysed	bioassayed
Kalotermes flavicollis	40	+	
Cryptotermes brevis	70	+	+
Mastotermes darwiniensis	30	+	+
Hodotermes mossambicus	40	+	
Zootermopsis angusticollis	40	+	
Reticulitermes flavipes	70	+	+
Reticulitermes santonensis	70	+	+
Heterotermes indicola	120	+	
Schedorhinotermes lamanianus	60	+	
Coptotermes formosanus	70	+	+
Coptotermes acinaciformis	80	+	+
Nasutitermes nigriceps	60	+	
Nasutitermes exitiosus	70	+	+
Trinervitermes trinervoides	30	+	
Macrotermes subhyalinus	40	+	

The methodology employed in the choice tests was that used by Reinhard et al. *supra*. In these experiments the termites were housed in a suitable container with access via a silicon tube to a foraging arena. In each

experiment two semicircles of moist filter paper (2.5cm in diameter) were placed close beside each other in the arena. One of the two semicircles was randomly chosen for application of one of the 25µl aliquots of labial gland extract and then moistened with water. The other semicircle was just moistened. Feeding in termites is expressed by gnawing behaviour, which can be easily recognised by the hypognathous head positions wherein the termites bore their mandibles into the food and wriggle their heads trying to tear off little pieces, which they can then transport back to the nest.

The distribution of the first 20 gnawing/feeding termites on the semicircles was registered. For example, it was observed that 19 of 20 *Mastotermes darwiniensis* termites responded by gnawing and eating the filter paper treated with one equivalent of its labial gland secretion while only one termite responded to the control. Similarly, 18 of 20 *C. acinaciformis* termites responded by gnawing and eating the filter paper treated with 2.5 equivalents of its labial gland secretion while 2 responded to the control. A further important observation was that termites of selected species also responded strongly in the bioassay to labial gland secretion from an unrelated species. For instance, *C. acinaciformis* termites responded to a test paper treated with one equivalent of *M. darwiniensis* gland secretion while *M. darwiniensis* termites responded to a test paper treated with 2.5 equivalents of *C. acinaciformis* gland secretion. These results demonstrate that the labial gland extract is a non-specific feeding stimulant for termites. The results are summarised in Table 2.

Table 2: Natural lures

termite species responding to lure	origin of labial gland extracts	quantity of extract (gland equivalents)	response
M. darwiniensis	*M. darwiniensis*	1	+++
	C. acinaciformis	2.5	+++
C. acinaciformis	*M. darwiniensis*	1	+++
	C. acinaciformis	2.5	+++

An analysis of the labial gland extract shows that para-hydroquinone is present at low levels, usually less than 10^{-10} grams per gland. Presumably β-arbutin is broken down enzymatically into para-hydroquinone and glucose during release of the termite's saliva, hence it was postulated that para-hydroquinone was the principal chemical feeding stimulant.

Example 2 - Synthetic Compounds as Termite Attractants

Feeding choice tests were conducted with para-hydroquinone (HQ) and a number of related chemical substances in the manner described above in Example 1. The experimental data is summarised in Table 3.

Table 3: Synthetic lures

termite species responding to lure	compound	quantity in lure [ng]	response
M. darwiniensis	p-hydroquinone	5	+++
	quinhydrone	5	++
	catechol	5	-/+
	resorcinol	5	+
	phloroglucinol	5	-/+
	4-methoxyphenol	5	+
	methoxyhydroquinone	5	+
	1,4-dimethoxybenzene	5	++
	toluhydroquinone	5	-
C. acinaciformis	p-hydroquinone	1	+++
	quinhydrone	5	++
	catechol	5	++
	resorcinol	5	++
	phloroglucinol	5	++
	4-methoxyphenol	5	+
	methoxyhydroquinone	5	+
	1,4-dimethoxybenzene	5	+
	toluhydroquinone	5	-
S. actuosus	p-hydroquinone	5	+++
C. brevis	p-hydroquinone	5	+++
N. exitiosus	p-hydroquinone	5	+++
R. santonensis	p-hydroquinone	5	+++
R. flavipes	p-hydroquinone	5	+++
C. formosanus	p-hydroquinone	5	+++

When synthetic lures were tested, none of the principal labial gland constituents (glucose, inositols, β-arbutin) elicited any feeding stimulation, except at unnaturally high concentrations where they probably served a nutritional role as food supplements. However HQ elicited feeding stimulation at natural trace levels in the laboratory bioassays. For instance the threshold for attraction was 5 nanograms HQ (50 picomoles) for *M. darwiniensis* and 100 picograms HQ (1 picomole) for *C. acinaciformis*. Thus, there are different lower thresholds of feeding stimulation for different termite species.

Synthetic compounds somewhat related in molecular structure to para-hydroquinone also elicited feeding responses from *M. darwiniensis* and *C. acinaciformis* in the laboratory bioassays. These compounds include quinhydrone, catechol (1,2-dihydroxybenzene), resorcinol (1,3-dihydroxybenezene), phloroglucinol (1,3,5-trihydroxybenzene), 4-methoxyphenol, methoxyhydroquinone (2,5-dihydroxyanisole) and 1,4-dimethoxybenzene.

<u>Example 3 - Mode of Attraction</u>

The mode of attraction of termites to the para-hydroquinone source may well include both olfactory and gustatory stimulation. The attractivity of para-hydroquinone (HQ) over distance (olfactory perception) was tested both in empty and sand-filled plastic arenas (ID 14.5 cm, height 1 cm, covered with a glass plate), which were attached via a silicone tube to the housing container of the termites. Tests were carried out with *M. darwiniensis* and *C. acinaciformis*. Per test, two treated filter papers (25ng - 25μg HQ and water as control, respectively) were placed in opposite positions in the arenas. The direction of the tunnel/galleries built and the behaviour of a foraging termites in reference to the position of the filter papers was evaluated. In all tests both termite species built tunnels/galleries in direction to the HQ-treated filter paper, never towards the control filter paper. When foraging the termites usually walked

slowly in a zigzag way, but when in proximity of the HQ-source (ca. 5-6 cm), their behaviour changed suddenly: they walked straight and fast to the treated filter paper. Based on these observational data we concluded that the vapour of HQ creates an "active space" of several centimetres, which once perceived directs the termites towards the source of the vapour by the concenctration gradient. This active space did not get larger with increased HQ-concentration.

DATED this 22nd day of December 1998.
COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION

By its Patent Attorney
GRIFFITH HACK